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(21) International Application Number: PCT/GB93/02644 (22) International Filing Date: 23 December 1993 (23.12.93) (30) Priority Data: 9227134.5 31 December 1992 (31.12.92) GB (71)(72) Applicants and Inventors: FULLER, Jess, Paul [GB/GB]; Flat 7, 35 Nottingham Road, Ashby-de-la-Zouch, Leicestershire LE65 1DL (GB). CLAYSON, Tony [GB/GB]; 9 Leicester Road, Ashby-de-la-Zouch, Leicestershire LE65 1DA (GB). KNIGHTS, Anthony, James [GB/GB]; 37 Pennard Road, Kittle, Bishopston, Swansea SA3 3JY (GB). (74) Agent: SKINNER, Michael, Paul; Swindell & Pearson, 48 Friar Gate, Derby DE1 1GY (GB).		(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: CELL SUPPORT STRUCTURES (57) Abstract A cell support structure (10) for immobilizing biological cells, comprising silicone foam rubber. The structure (10) may be of any suitable form, for instance granular, sheet, bead, tube, chip, strand, pad or block. The structure (10) naturally adsorbs biological cells on its surfaces, thereby immobilizing them but leaving them free to partake in desired reactions, for instance the production of biological compounds and fermentation reactions. The physical properties of the support structure (10), such as porosity and density are engineered to suit particular applications. Moreover, agents may be provided on the surface of the structure (10) to interact with the cells and other ingredients involved in the reaction. Once the reactions are complete, the structures (10), to which the cells are generally still attached, are filtered or otherwise separated from the reaction mixture. The desired product is then separated from the reaction mixture. The support structure (10) can then be cleaned of cells if desired and sterilised for re-use using techniques such as autoclaving, without substantial deterioration of the structures (10).		

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Cell Support Structures

The present invention relates to cell support structures, particularly but not exclusively to cell support structures for immobilizing cells.

The immobilization of biological cells by loading them onto a support structure is a technique often used by scientists in the production of important biologically derived products. The technique offers numerous advantages over free cell, liquid phase systems, including permitting increased ease of separation of the cells from the reaction medium, increased throughput flow rates in continuous reaction systems, increased cell density per unit volume of reaction medium, and the production of a relatively cell-free, product containing medium which in turn facilitates downstream processing.

Conventional immobilization materials suffer from several disadvantages. Some materials, such as synthetic polymers are relatively expensive and harmful, often causing a decrease in cell viability and/or product activity. Other materials used such as Agar, are mechanically soft, unstable and also cause cell viability problems. Calcium Alginate is currently one of the more popular materials used, but suffers from being relatively unstable and mechanically weak, and thereby prone to

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structural collapse during use, and is also susceptible to chemical attack.

It is an object of the present invention to obviate or mitigate these disadvantages.

According to the present invention there is provided a resilient cell support structure comprising silicon or a derivative thereof.

The structure may be of any suitable configuration, for instance beads, blocks, sheets, pads, chips, strands, tubes or granules. The structure may be formed or adapted to be locatable in or around and/or connectable to other component(s), for instance a reaction vessel or the like.

Preferably the structure is porous, for instance, the structure may be a sponge or foam. The porosity and/or density of the structure is preferably controlled according to the intended application. The porosity and/or density may be engineered by adding one or more additives to the structure, preferably substantially inert additives. Metal powder, such as stainless steel powder may be added to provide a relatively dense structure. Alternatively or additionally the porosity

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and/or density may be engineered by controlling aeration of liquid structure material during manufacture of the structure.

Preferably one or more substances may be added to give the structure particular surface properties. The or at least one of the substances may cooperate with biological cells.

Preferably the structure comprises silicone rubber.

Preferably the structure is re-usable.

The invention also provides a method of immobilizing cells the method comprising introducing a cell or cells to a resilient support structure comprising silicon or a derivative thereof.

The structure used may be of any suitable configuration, for instance block, sheet, pad, chip, strand, tube or granule. The structure may be formed or adapted to be locatable in or around and/or connectable to other component(s), for instance a reaction vessel or the like.

Preferably a culture of cells is grown on the

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support structure, preferably in conditions favouring growth. A plurality of cell cultures of different types of cells may be grown on a support structure.

Preferably the cells are introduced and/or grown on a porous structure, preferably in favourable conditions of acidity or alkalinity. The structure may be a sponge or foam. The growth conditions may be controlled to enable the extent of colonisation of the structure by the cells to be controlled.

The porosity and/or density of the structure is preferably controlled according to the intended application. The porosity and/or density may be engineered by incorporating one or more additives to the structure, preferably inert additives. Metal powder such as stainless steel powder may be added to provide a relatively dense structure. Alternatively or additionally the porosity and/or density may be engineered by controlling aeration of liquid structure material during manufacture of the structure.

Preferably one or more substances may be added to give the structure particular surface properties. The or at least one of the substances may cooperate with biological cells.

Preferably the cell(s) are introduced to a structure of silicone rubber.

Preferably the structure is sterilised before use. Preferably the structure is re-usable, and may be re-sterilised for re-use, once the desired reaction is complete.

According to a further aspect of the present invention there is provided a reaction system comprising a resilient cell support structure comprising silicon or a derivative thereof located within a reaction medium comprising a reactant, the support structure having cells located thereon in use, to enable or facilitate the desired reaction. The structure may have any of the features set forth above.

An embodiment of the present invention will now be described by way of example only, with reference to the accompanying drawing which is a diagrammatic representation of a cross-section through a cell support structure according to the present invention.

A cell support structure 10 for immobilizing biological cells, comprises silicone foam rubber. The structure 10 may be in any form convenient for the

particular intended use, for example granular (see drawing), sheet, bead, tube, chip, strand, pad or block form.

The structure 10 may also be formed or shaped to locate on or in or to connect to any other component of a reaction system in which it is to be used, for instance the or each structure may be shaped for assembly into the structure of a bioreactor.

During manufacture of the structure 10, if it is desirable, the porosity and/or density of the resultant structure 10 can be engineered. This may be done by selectively setting the environmental conditions of manufacture and/or incorporating inert additives into the structure 10. For instance dense particles such as stainless steel powder may be added to produce relatively dense structures. Alternatively, or in addition, the porosity may be increased (and density decreased) by increasing aeration of the liquid rubber during manufacture of the structures 10. This is important, since where the cells to be used are relatively large, then it is desirable to produce a structure 10 with relatively large pores 12, to accommodate the cells. In contrast when immobilizing cells that are relatively small, it is desirable to use a structure having pores 12

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of relatively small size, thus maximizing the available surface area within the structure 10 to which the cells can attach. If the pores 12 are unduly large, then there will be considerable stacking of cells on the available surfaces. This stacking prevents cells cloaked by other cells from interacting with the reaction mixture efficiently. What is more, the available reaction surface will be relatively small. These conditions provide for poor efficiency.

The density is preferably increased as above when the reaction produces gases or otherwise involves gases, which aerate the reaction mixture and would tend to increase the buoyancy of the structure 10 through the adherence of bubbles to the structure 10. Increasing the density as necessary will ensure that the structures 10 and cells attached thereto remain in the reaction mixture to give maximum reaction exposure, and not allow them to float to and remain on the surface of the reaction mixture where the cells will not be involved satisfactorily in the desired reaction. This is relevant to fermentation reactions, where significant volumes of carbon dioxide are often produced.

As mentioned, the structures 10 are intended primarily for use in reaction systems comprising

biological cells (Bioreactors) to immobilize the cells.

The immobilized cells are physically confined or localised on the surfaces of the structure 10, including surfaces defining pores within the structure 10. The structure 10 may be manufactured to comprise agents on the surface thereof which facilitate retention of the cells and/or the desired reaction. The cells retain their catalytic properties, preferably along with their viability, and can be used repeatedly and continuously. It is to be appreciated that there may be some leakage of cells from the structure 10. This leakage may or may not be significant, depending upon the particular reaction involved. It is possible to use the structures 10 as a means of feeding cells into a reaction medium, in particular reaction conditions.

The silicone structure 10 immobilizes the cells by adsorbing the cells onto its surfaces, which are inert. It may be termed a "natural" immobilization since the cells have a natural tendency to coat the surfaces and line the pores 12 of the structure, enabling maximum cell biomass to be loaded onto the structures 10. This in turn allows for maximum biocatalytic activity. However the efficiency of this form of immobilization is found to be affected by the environmental conditions, and often an

optimum value for various factors such as acidity and alkalinity must be found in order to maximise the adsorption of the cells on to the support structure 10. Adsorption immobilization has the advantage of being very gentle and passive enabling cells to generally retain their viability.

Thus, a culture of the relevant cells is first grown on sterile support structures 10 in conditions for optimum growth (which may be different from the normal operating conditions in the bioreactor). The resilient nature of the structures 10 permits considerable cell loading, without collapse or disintegration of the structure 10. The foam nature of the structure 10 increases the surface area available for colonisation, per unit volume of material. It is to be appreciated that the density of colonisation (loading) of the structures 10 can be predetermined by setting the growth conditions. For example, one essential nutrient or condition may be limiting. Furthermore, it is also possible, if desired, to grow cultures of different cells on the structures 10. Once the culture 10 has been grown to the desired density on the support structure 10, the cell-loaded structures 10 can be introduced to the reaction medium, which may be in a bioreactor as will be described.

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Thus, in use, cell-loaded structures 10 are introduced into the reaction medium containing the ingredients necessary for the cells to perform and produce the desired product(s). The conditions within the bioreactor may be different to those in which the structure 10 was loaded. The bioreactor conditions are then maintained to allow the cells to react with the surrounding medium to produce the required products.

Once the reaction is complete, then the cell-loaded structures 10 may be filtered, gently centrifuged or otherwise simply removed from the reaction medium, which now contains the desired product. Simple filtering techniques are sufficient to leave a relatively cell free, product-containing medium. The product-containing medium may contain some free cells leaked from the structures 10 which may be removed using further purification techniques if desired. The product can then be isolated from the medium using conventional techniques.

The support structures 10 can be re-used. They can be cleaned of cells by using a variety of conventional techniques, including chemical and mechanical cleaning. Alternatively, the cells may be allowed to remain, for re-use. The robust, durable and resilient properties of

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the silicone rubber foam enable it to withstand pressures from high cell packing within the pores, shear and impact with moving objects, for example an impeller which may be employed during the reaction. The structures 10 can be mechanically filtered out of the product-containing medium, under pressure if necessary, without harm. The structures 10 can be cleaned in steam, for example superheated steam (126°C) for an hour or more, and are also resistant to deterioration in an autoclave. Chemical cleaning can also be employed, including solvents such as ethanol, acid bleaching and suitable acid or alkali cleaning liquids can be used without detriment. This enables the support structures 10 to be effectively and efficiently cleaned to a condition for re-use.

The non-contaminating and inert nature of the support structures 10 enables them to be used in the production of proteins and other materials for use in the treatment of human and animal bodies, for example, in the production of proteins such as somatostatin, human growth hormone etc. The structures 10 are also particularly useful for purifying liquids, for instance water. Loaded structures 10 can be introduced into contaminated water. The cells, chosen according to a particular application, take-up the contaminants, reduce them to a non- or less-hazardous material, or otherwise remove or reduce the

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contamination, to leave purified or part purified water. This is particularly useful in the treatment of sewage waste. The structures 10 are also expected to be useful in the fermentation industry, allowing yeast cells to be attached to the structures 10 throughout the fermentation process and removed with the structures 10, so that the conventional problems associated with clouding of the fermentation mixture are obviated or mitigated.

It has been found that the silicone structure is non-toxic to and biocompatible with living cells, and also tends to encourage cell adhesion to, and colonisation of, the pore surfaces of the structure 10. Experiment has shown that brewing yeast, Saccharomyces cerevisiae, enjoys the above benefits, which indicate that both bacteria and moulds, as well as plant and animal cells are expected to be efficiently immobilizable on the structures 10.

The resilient nature of the structure 10 also enables substantial accumulation of cells, without disintegration of the structure 10. This has been a particularly notable disadvantage in conventional systems. Furthermore, the silicone structure does not appear to inhibit cell action, or function.

Whilst endeavouring in the foregoing specification to draw attention to those features of the invention believed to be of particular importance it should be understood that the Applicant claims protection in respect of any patentable feature or combination of features hereinbefore referred to and/or shown in the drawings whether or not particular emphasis has been placed thereon.

CLAIMS

1. A resilient cell support structure comprising silicon or a derivative thereof.
2. A resilient cell support structure comprising silicon or a derivative thereof located within a reaction medium comprising a reactant, the support structure having cells located thereon in use, to enable or facilitate the desired reaction.
3. A structure according to claim 1 or claim 2, in which the structure is of any suitable configuration.
4. A structure according to claim 3, in which the structure is in the form of beads, blocks, sheets, pads, chips, strands, tubes or granules.
5. A structure according to any preceding claim, in which the structure is formed or adapted to be locatable in or around and/or connectable to another component.
6. A structure according to claim 5, in which the structure is locatable in and/or connectable to a reaction vessel.

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7. A structure according to any preceding claim, in which the structure is porous.

8. A structure according to any preceding claim, in which the structure is a sponge or foam.

9. A structure according to claim 7 or claim 8, in which the porosity of the structure is controlled according to the intended application.

10. A structure according to claim 9, in which the porosity is engineered by adding one or more additives to the structure.

11. A structure according to any preceding claim, in which the density of the structure is controlled according to the intended application.

12. A structure according to claim 11, in which the density is engineered by adding one or more additives to the structure.

13. A structure according to any of claims 10 to 12, in which the or each additive is inert or substantially inert.

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14. A structure according to any of claims 11 to 13, in which metal powder is incorporated in the structure.

15. A structure according to claim 14, in which stainless steel powder is added to provide a relatively dense structure.

16. A structure according to any of claims 7 to 15, in which the porosity and density are engineered by controlling aeration of liquid structure material during manufacture of the structure.

17. A structure according to any preceding claim, in which a substance is added to give the structure desired surface properties.

18. A structure according to claim 17, in which the substance cooperates with biological cells.

19. A structure according to any preceding claim, in which the structure comprises silicone rubber.

20. A structure according to any preceding claim, in which the structure is re-usable.

21. A method of immobilizing cells the method

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comprising introducing a cell or cells to a resilient support structure comprising silicon or a derivative thereof.

22. A method according to claim 21, in which the structure used is of any suitable configuration.

23. A method according to claim 22, in which the structure used is in the form of a block, sheet, pad, chip, strand, tube or granule.

24. A method according to any of claims 21 to 23, in which the structure is formed or adapted to be locatable in or around and/or connectable to other component(s).

25. A method according to claim 24 in which the structure is formed or adapted to be locatable in and/or connectable to a reaction vessel.

26. A method according to any of claims 21 to 25, in which a culture of cells is grown on the support structure.

27. A method according to claim 26, in which cells are grown in conditions favouring growth.

28. A method according to claim 26 or claim 27, in which a plurality of cell cultures, perhaps of different cells, are grown on a support structure.

29. A method according to any of claims 26 to 28, in which cells are introduced and/or grown on a porous structure.

30. A method according to any of claims 26 to 29, in which the cells are introduced and/or grown in favourable conditions of acidity or alkalinity.

31. A method according to any of claims 21 to 30, in which the structure is a sponge or foam.

32. A method according to claim 30 or claim 31, in which the growth conditions are controlled to enable the extent of colonisation of the structure by the cells to be controlled.

33. A method according to any of claims 29 to 32, in which the porosity of the structure is controlled according to the intended application.

34. A method according to claim 33, in which the porosity is engineered by incorporating one or more

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additives to the structure.

35. A method according to any of claims 21 to 34, in which the density of the structure is controlled according to the intended application.

36. A method according to claim 35, in which the density of the structure is engineered by incorporating one or more additives to the structure.

37. A method according to any of claims 34 to 36, in which the or each additive is inert or substantially inert.

38. A method according to any of claims 35 to 37, in which at least one of the additives comprises metal powder.

39. A method according to claim 38, in which stainless steel powder is added to provide a relatively dense structure.

40. A method according to any of claims 29 to 39, in which the porosity and density are engineered by controlling aeration of liquid structure material during manufacture of the structure.

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41. A method according to any of claims 21 to 40, in which a substance is added to give the structure desired surface properties.

42. A method according to claim 41, in which the substance cooperates with biological cells.

43. A method according to any of claims 21 to 42, in which the cell(s) are introduced to a structure of silicone rubber.

44. A method according to any of claims 21 to 42, in which the structures are sterilised before use.

45. A method according to any of claims 21 to 42, in which the structures are re-usable.

46. A method according to claim 45, in which the structures can be re-sterilised for re-use.

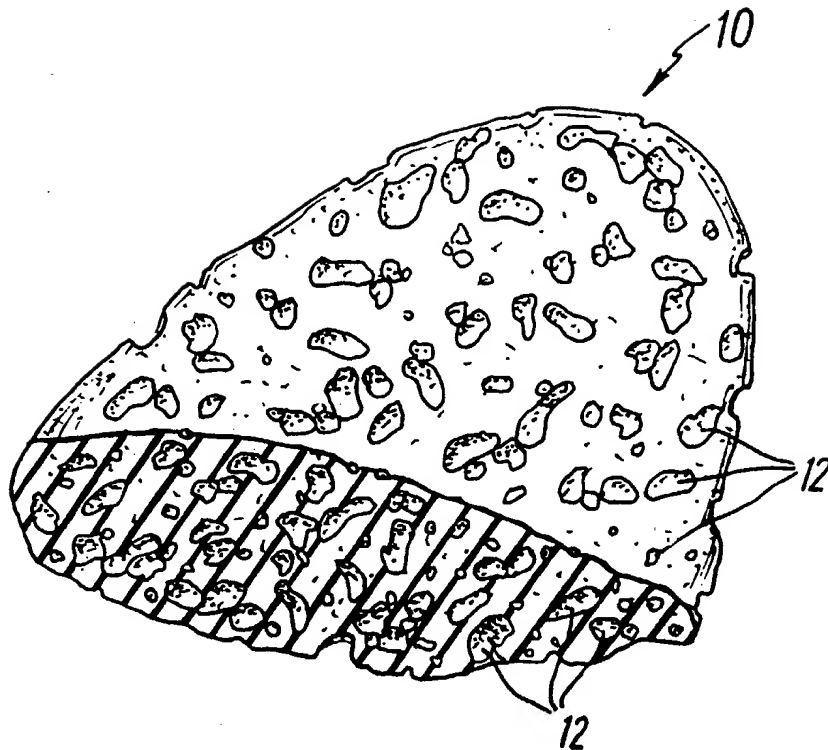
47. A structure substantially as hereinbefore described with reference to the accompanying drawings.

48. A method substantially as hereinbefore described with reference to the accompanying drawings.

49. A reaction system substantially as hereinbefore described with reference to the accompanying drawings.

50. Any novel subject matter or combination including novel subject matter disclosed, whether or not within the scope of or relating to the same invention as any of the preceding claims.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 93/02644

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N5/02 C12N11/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>ENZYME AND MICROBIAL TECHNOLOGY vol. 10, no. 9, September 1988, HAYWARDS HEATH, ENGLAND pages 518 - 523 ORIEL 'IMMOBILIZATION OF RECOMBINANT ESCHERICHIA COLI IN SILICONE POLYMER BEADS' see the whole document ---</p>	<p>1-13, 16-37, 40-49</p>
X	<p>FR,A,2 522 014 (INSTITUT PASTEUR ET CNRS) 26 August 1983 see the whole document ---</p>	<p>1-13, 16-37, 40-49</p>

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

Inter. Application No

PCT/GB 93/02644

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE WPI Section Ch, Week 8838, Derwent Publications Ltd., London, GB; Class A96, AN 88-268198 'BASE FOR CELL CULTURE-COMPRISES COMPLEX OF HIGH MOL.WT.POLYMER WITH INORGANIC SUBSTANCE' & JP,A,63 196 281 (SUMITOMO ELEC IND KK) 15 August 1988 see abstract</p> <p style="text-align: center;">---</p>	<p>1-13, 16-37, 40-49</p>
X	<p>BIO/TECHNOLOGY vol. 4, no. 6 , June 1986 , NEW YORK,USA pages 505 - 510 VAN BRUNT 'IMMOBILIZED MAMMALIAN CELLS:THE GENTLE WAY TO PRODUCTIVITY' see the whole document</p> <p style="text-align: center;">-----</p>	<p>1-13, 16-18, 20-37, 40-42, 44-46</p>

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 93/02644

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR-A-2522014	26-08-83	NONE	